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# Quantification of tissue properties in small volumes

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## ABSTRACT

The quantification of tissue properties by optical measurements will facilitate the development of noninvasive methods of cancer diagnosis and detection. Optical measurements are sensitive to tissue structure which is known to change during tumorigenesis. The goals of the work presented in this paper were to verify that the primary scatterers of light in cells are structures much smaller than the nucleus and then to develop an optical technique that can quantify parameters of structures the same size as the scattering features in cells. Polarized, elastic back-scattering was found to be able to quantify changes in scattering properties for turbid media consisting of scatterers of the size found in tissue.

## 1. INTRODUCTION

The ability to quantify local tissue optical properties could facilitate several applications of biomedical optics including cancer diagnosis, tissue identification during surgery, and dosimetry prior to photodynamic therapy. Tissue optical properties are commonly quantified using some of the following parameters; the distribution of angles through which light is scattered,  $P(\theta)$ , the scattering coefficient,  $\mu_s$ , the absorption coefficient,  $\mu_a$ , the anisotropy factor  $g$ , and the reduced scattering coefficient,  $\mu_s'$ . In a large, highly scattering, not too absorbing, homogenous volume, it is possible to determine  $\mu_s'$  and  $\mu_a$  using the diffusion approximation. Alternative to determining the optical properties, information about the size and concentration of scattering structures and about the concentration and types of absorbers may be determined. Knowledge of the effective scatterer size distribution can be used to calculate optical properties. Conversely, in many cases the optical properties can be used to calculate the size distribution of scattering centers assuming spherical, non-interacting scatterers<sup>1,2</sup>. This quantification of scatterer properties is particularly important in applications such as cancer detection, where changes in structure are known to correlate with disease.

In this paper we first present experimental data that measures the effective size of scatterers in tissue. For this work multicellular spheroids were used as an *in vitro* model of cellular tissue. Multicellular spheroids are spherical aggregates of tumor or normal cells and the extracellular matrix which they produce. They are a model system for the microenvironment of tumors and have been used to study the effects of microenvironmental stresses and cell-cell interactions on cellular proliferation, cellular viability, gene expression, metabolism, invasion, and response to numerous forms of cancer therapy<sup>3,4,5,6</sup>. Secondly, we describe a method for determining the effective scatterer size in dense scattering media containing a broad distribution of scatterer sizes. We have developed a fiber-optic probe that measures the polarization properties of elastically scattered light in a backscattering geometry.

## 2. METHODS

### 2.1 Polarized angular-dependent light scattering measurements

Polarized angular dependent light scattering measurements were made of MR1 cells. MR1 cells are a *ras* and *myc* transformed fibroblast cell line which are tumorigenic. Multicellular spheroids were initiated from exponentially-growing monolayer cultures essentially as described previously<sup>7</sup>. After making angular-dependent light scattering measurements on intact spheroids, the aggregates were dissociated to a single-cell suspension, allowing for measurement of exactly the same cell culture under aggregated and single-cell conditions.

For measurement of angular dependent light scattering, cells and spheroids were suspended in 50 ml of saline. The cell concentrations varied between  $9 \times 10^4$  and  $9 \times 10^5$  cells per ml. The experimental apparatus used for measuring angularly resolved light scattering has been described previously<sup>8</sup>. Briefly, an unpolarized HeNe laser (Uniphase, Inc.) was incident on a suspension of cells and the scattered light was measured with a photomultiplier tube (Hamamatsu, Inc.) which could be

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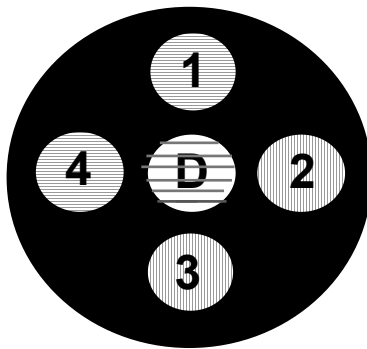
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rotated around the sample cell. To obtain the dynamic range necessary for the experiment, neutral density filters were placed in front of the laser. Two different sample cells were used. One contained a beam stop to reduce reflection from the glass container that can interfere with measurements of high angle scatter. The other sample cell consists of a simple glass cylinder on a base and was used for measuring scattering at angles of  $45^\circ$  and smaller. Data is usually obtained between  $\sim 6$  and  $172^\circ$ . The most reliably high quality data is obtained between  $15$  and  $160^\circ$ . Systematic errors due to stray light scattering are very difficult to avoid at angles greater than  $160^\circ$ . To assure that the system was giving accurate results, measurements of polystyrene spheres were compared to theory.

## 2.2 Polarized elastic scatter measurements in a backscattering geometry

We have further developed a fiber optic probe described previously<sup>9</sup>. All of the data presented here was performed using a probe with the configuration of Figure 1. The center fiber delivers broad band light through a linear polarizer. Fibers 1 and 4 collect light with the same polarization. Fibers 2 and 3 collect light polarized perpendicular to the incident light. The optical fibers have a core diameter of 200 microns and the center-to-center separation between the delivery fiber and the collection fibers is 550 microns. The collected light is dispersed and detected on a two dimensional CCD array. More recently we have developed a new probe, that contains fibers, D,1,4,3 as well as two addition fibers for making unpolarized measurements. The probe is 3 mm in diameter. It is quite small compared to a penny, as seen in Figure 2. Both probes have polarizers on the probe end that is in contact with the scattering medium. These polarizers are efficient from 500 – 700 nm, but do not polarize light with wavelengths longer than 950 nm. The spectral region between 965 and 1000 nm is used to correct for any differences in light collection efficiency of the detection fibers. All measurements are also referenced to a measurement of a spectrally flat material to account for the wavelength dependent properties of the probe, spectrograph and CCD array.

The fiber optic probe was used to make polarized backscattering measurements of several mixture of polystyrene spheres. Each polystyrene sphere mixture consisted of four sizes of polystyrene spheres. Each type of mixture was made in three concentrations with reduced scattering coefficients of 10.8, 16.2 and  $21.5 \text{ cm}^{-1}$ . We also performed Monte Carlo simulations using a modified version of our Monte Carlo code that implemented the procedures of Bartel and Hielscher<sup>10</sup> that track light polarization.



## 2. RESULTS

Figure 1. Left: End on view of the fiber geometry in the probe used for the measurements presented here. The light delivered by fiber D and collected by fibers 1 and 4 has the same polarization. Fibers 2 and 3 collect light polarized perpendicular to the polarization of the delivered light. Right: A picture of a penny and our recently designed fiber optic probe.

### 3. RESULTS

#### 3.1 The size of scattering centers in tissue

The angular dependence of scattering of light with a polarization parallel to the scattering plane is very sensitive to the size of the scattering centers. For Rayleigh scatterers, i.e. scatterers that have a diameter much less than the wavelength of light, there is a sharp dip in scattered light intensity at  $90^\circ$ . As the size of the scattering centers increases, the amount of scattering at  $90^\circ$  increases, the dip shifts to higher angle initially, and the difference between the scattering of light polarized parallel and perpendicular to the scattering plane decreases. This phenomenon is illustrated by Figure 2. Figure 3 presents the results of angular dependent scattering measurements of multicellular spheroids and of cells dissociated from the multicellular spheroids. There is a clear dip near  $95^\circ$  in the intensity of scattered light polarized parallel to the scattering plane. Comparison with Figure 2 indicates that the average effective radius of the scattering centers is around 0.4 microns. Also, from Figure 3 it is clear that light scattering from multicellular spheroids and from cells dissociated from the spheroids is very similar.

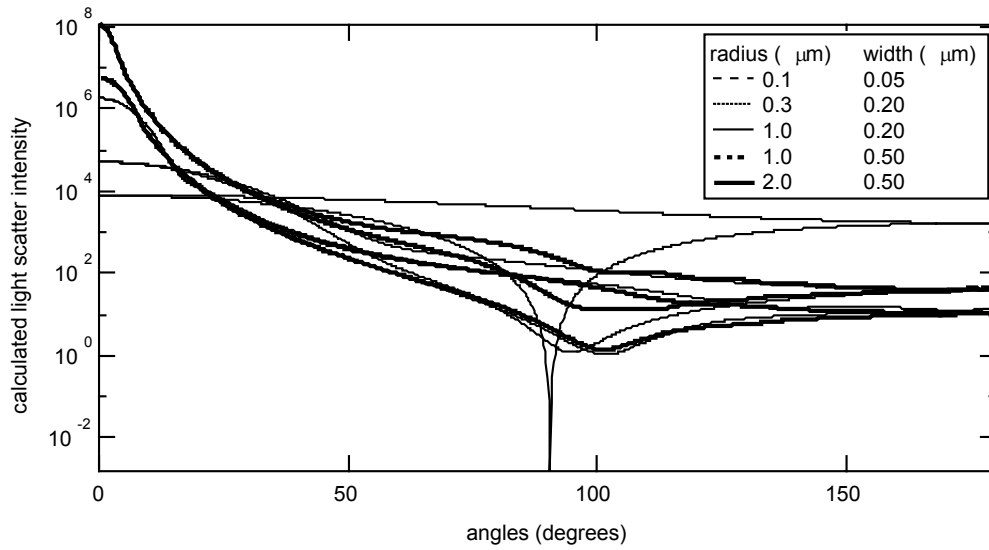


Figure 2. Mie theory calculations of scattering with polarization parallel and perpendicular to the scattering plane. For each pair of curves, the top curve represents scattering of light polarized perpendicular to the scattering plane and the bottom curve represents scattering of light polarized parallel to the scattering plane. For each calculation a log-normal distribution of scatterers of index 1.39 in a medium of index 1.33 was assumed. The left column of the legend is the average radius while the right column is a measure of the width of the distribution.  $\lambda = 633 \text{ nm}$ .

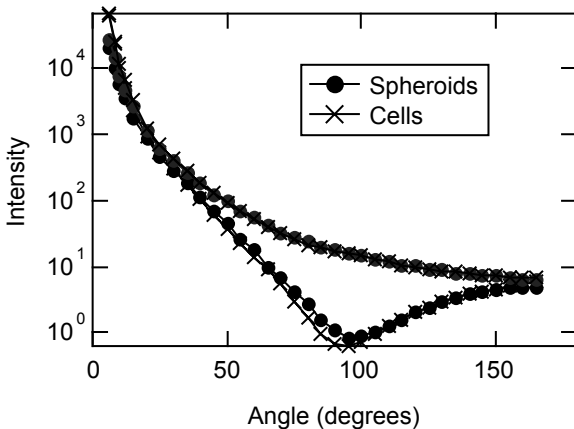


Figure 3. Results of angular dependent measurements of light scattering from suspensions of multicellular spheroids and of cells dissociated from the multicellular spheroids. For each set of curves, the top curve was measured with the incident and detected light polarized perpendicular to the scattering plane and the bottom curve was measured with the incident and detected light polarized parallel to the scattering plane.

### 3.2 Polarized, fiber-optic backscattering measurements

White light was delivered to a polystyrene sphere suspension with an average scatterer diameter of 224.2 nm by fiber D of the fiber optic probe shown schematically in Figure 1. The light intensity collected by fibers 1 and 3 divided by a measurement of a spectrally flat material are shown in Figure 4. The two curves have been scaled so that the intensity between 965 and 1000 nm is the same. The intensity of light collected by fiber 1 is greater than the intensity of light measured by fiber 4. This result is due to the fact that for small scattering particles, scattering at angles near  $90^\circ$  is more likely if the light is polarized perpendicular to the scattering plane than if it is polarized parallel to the scattering plane. When a photon travels from fiber D to fiber 1, the polarization is roughly perpendicular to the scattering plane. In contrast, for most trajectories between fiber D and fiber 4, the polarization is roughly parallel to the scattering plane. Consequently, scattering is more likely on the trajectories between fibers D and 1 than on the trajectories between fibers D and 4 and more light is collected by fiber 1 than by fiber 4. When the scattering media contains larger scattering centers, the difference in light intensity collected by fibers 1 and 4 is smaller because the difference in scattering properties for the two polarizations decreases. Therefore, the ratio of light intensities collected by fiber 1 and fiber 4 is sensitive to particle size.

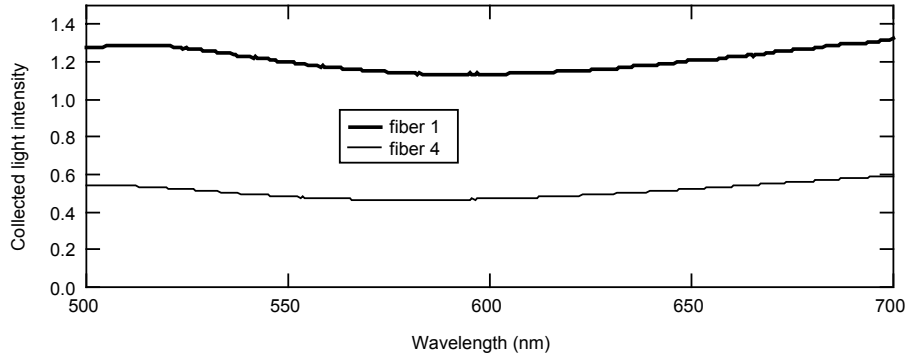


Figure 4. Results of polarized elastic-scattering measurements of a suspension of polystyrene spheres with an average diameter of 224 nm.

In our measurements of polystyrene sphere suspensions, we have found that  $I_1/I_4$  is sensitive to scatterer concentration as well as to scatterer size. Therefore, we need to measure another parameter.  $I_1/I_3$  should be quite sensitive to scatterer concentration, since one fiber measures polarization that is preserved and the other measures the perpendicular polarization. As scatter concentration increases, more scattering events will occur on trajectories between the delivery and collection fibers and therefore the light will be depolarized more.

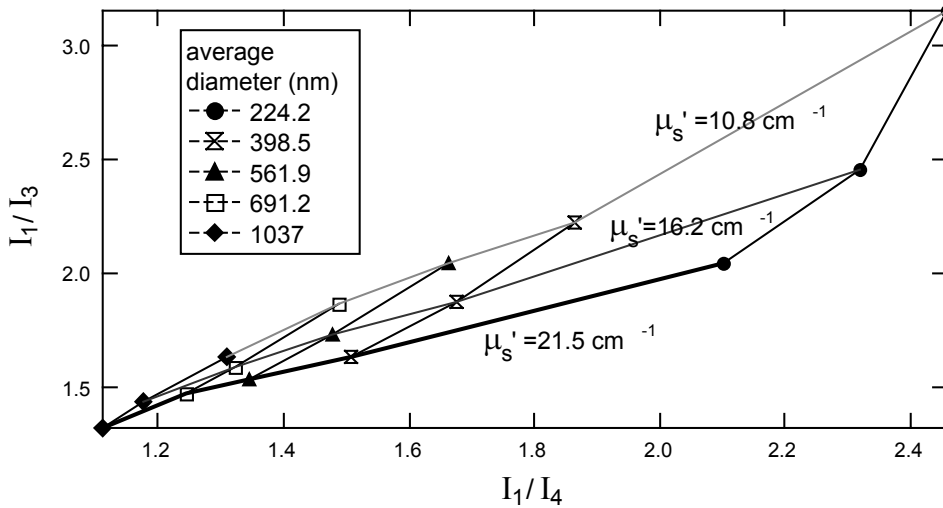


Figure 5. The ratio of light intensities at 575 nm measured by fibers 1 and 3 versus the ratio of light intensities at 575 nm measured by fibers 1 and 4. The thin gray line, the thin solid black line and the thick solid black line connect measurements of tissue phantoms with  $\mu_s' = 10.8$ ,  $16.2$  and  $21.5 \text{ cm}^{-1}$ , respectively. The dashed lines each connect measurements made of tissue phantoms with a particular average scatterer size.

We have made measurements of  $I_1/I_3$  and  $I_1/I_4$  for polystyrene suspensions with a wide variety of scatterer sizes and concentrations. The results at 575 nm are shown in Figure 5, where the x-axis is  $I_1/I_3$  and the y-axis is  $I_1/I_4$ . As scatterer size increases, the ratio of  $I_1/I_4$  decreases as expected.  $I_1/I_4$  also decreases with increasing scatterer concentration. Like  $I_1/I_4$ ,  $I_1/I_3$  decreases with increasing scatterer concentration and decreases with increasing scatterer size. Because the dependencies of  $I_1/I_3$  and  $I_1/I_4$  on scatterer size and concentration are different we can use these parameters to determine effective scatterer size and concentration.

Polystyrene spheres have an index of refraction of 1.59 and were immersed in water. The refractive indices in tissue have a much smaller refractive index. We have used Monte Carlo simulations of photon transport to investigate how this change in refractive index affects the independent measurements of scatterer size and concentration. The change of scatterer refractive index does not have a large effect although it appears to make the resolution for measuring the average size of suspensions with large scatterers slightly better.

Finally, we have made measurements of dense suspensions of mammalian cells (MR1) and compared our results to the Monte Carlo simulations. We find that the average effective radius of scattering particles is about  $0.5 - 1.0 \mu\text{m}$ .

#### 4. DISCUSSION AND CONCLUSIONS

The epithelium, where most cancers originate, is a predominantly cellular structure with very little extracellular material<sup>11</sup>. Therefore, methods developed for measuring structural changes in cells should be applicable to *in vivo* epithelial tissue. Section 3.1 demonstrates that the cellular structures scattering light are predominantly small - significantly smaller than the nucleus. This result is corroborated by our polarized fiber optic backscattering measurements as well as by earlier work<sup>12</sup>. In addition, Kumar and Schmitt have demonstrated that the light scattering properties of tissue can be modeled by a log-normal distribution of spheres with an average diameter around  $0.4 \mu\text{m}$ <sup>13</sup>.

The ability to examine a small volume of tissue is a desirable characteristic of a noninvasive measurement system because the epithelium is thin and the early cellular changes may be localized. Another desirable characteristic of a non-invasive measurement system is sensitivity to the size structures in epithelial tissue that scatter light. Section 3.2 demonstrates that polarized elastic backscattering is sensitive to scatterers of roughly the same size as those found in tissue. Furthermore, the technique can separately quantify two parameters, effective scatterer size and concentration. We have used Monte Carlo simulations to investigate the depth probed by this technique (data not shown). The depth probed depends strongly on the light absorption properties of tissue. Depending on the wavelength used for the calculations of scatterer size and concentration and on the concentration of hemoglobin it should be possible to vary the depth above which half of all scattering events occur from 230 to  $435 \mu\text{m}$ . If the separation between the source and detector fiber can be shortened, the depth probed can be reduced further in order to only interrogate the epithelial layer.

Another technique being developed by Depeursinge and coworkers to interrogate small volumes, measures elastic scattering using collection fibers at multiple distances from the source fiber<sup>14</sup>. Using tissue phantoms they have demonstrated that they can measure  $\mu_a$ ,  $\mu_s'$  and a parameter of the phase function  $\gamma = (1-g_2)/(1-g_1)$ , where  $g_1$  and  $g_2$  are the first two moments of the phase function. The largest source detector separation in the probe designed by Depeursinge and coworkers is greater than  $550 \mu\text{m}$  and consequently a deeper tissue depth will be probed than with our technique.

The similarity in results when multicellular spheroids and a suspension of cells were measured demonstrates that it is internal cellular structures that are the dominate light scatterers and that changes in cell contact and cell shape have very little effect on light scattering. The exact cellular structures that scatter light are not known. They could be cytoplasmic features or structural inhomogeneities in the nucleus which are caused by chromatin clumping. Two dimensional finite difference time domain simulations have shown that larger index of refraction variations in the nucleus, that simulate the more heterogeneous chromatin structure of a dysplastic cell, cause significant changes in light scattering properties. Changes in nucleoskeletal structure occur very early in tumorigenesis, possibly even due to the mutation of a single gene<sup>15</sup>.

## 5.0 ACKNOWLEDGEMENTS

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## 6.0 REFERENCES

1. H. Jiang, J. Pierce, J. Kao, and E. Sevcik-Muraca (1997) "Measurement of particle-size distribution and volume fraction in concentrated suspensions with photon migration techniques," *Appl. Opt.*, **36**, 3310-3318.
2. H. Jiang (1998) "Enhanced photon-migration methods for particle sizing in concentrated suspensions," *AIChE Journal*, **44**, 1740-1744.
3. R. M. Sutherland "Cell and environment interactions in tumor microregions: The multicell spheroid model," *Science* **240**, 177-184 (1988).
4. J. P. Freyer, "Spheroids in Radiobiology Research" In: *Spheroid Culture in Cancer Research*, Bjerkvig R, ed. (CRC Press), pp. 217-275 (1992).
5. W. Mueller-Klieser "3-dimensional cell cultures: from molecular mechanisms to clinical applications." *Am. J. Physiol. Cell Physiol.* **42**, C1109-C1123 (1997).
6. L. A. Kunz-Schughart, M. Kreutz, R. Knuechel "Multicellular spheroids: a 3-dimensional in vitro culture system to study tumor biology," *Int. J. Exp. Pathol.* **79**, 1-23 (1998).
7. J.P. Freyer "Mitochondrial function of proliferating and quiescent cells isolated from multicellular tumor spheroids," *J. Cell. Physiol.*, **176**, 138-149 (1998).
8. J. R. Mourant, J. P. Freyer, A. H. Hielscher, A. Eick, D. Shen, T. M. Johnson, "Mechanisms of light scattering from biological cells relevant to noninvasive optical-tissue diagnosis," *Appl Opt.* **37**: 3586 – 3593 (1998).
9. T. M. Johnson, and J. R. Mourant, "Polarized wavelength-dependent measurements of turbid media" *Opt. Express* **4**, 200 – 216 (1999).
10. S. Bartel, and A. H. Hielscher "Monte Carlo simulation of the diffuse backscattering Mueller matrix for highly scattering media," *Appl. Opt.* **39**, 1580 – 1588 (2000).
11. E. N. Marieb, *Human Anatomy and Physiology*, Benjamin Cummings 1992 pg. 104.
12. J. R. Mourant, M. Canpolat, C. Brocker, O. Esponda-Ramos, T. Johnson, A. Matanock, K. Stetter, J. P. Freyer, "Light scattering from cells: the contribution of the nucleus and the effects of proliferative status," *J. Biomed. Optics* **5**, 131 - 137 (2000).
13. J. M. Schmitt and G. Kumar, "Optical scattering properties of soft tissue: a discrete particle model" *Appl. Opt.* **37**:2788 – 2796 (1998).
14. F. Bevilacqua, D. Piguet, J. D. Gross, B. J. Tromberg, C. Depeursinge "Monte Carlo study of diffuse reflectance at source-detector separations close to one transport mean free path," *Appl. Opt.*, **38**, 4939-4950 (1999).
15. A. H. Fischer, D. N. Chadee, J. A. Wright, T. S. Gansler, J. R. Davie, "Ras-associated nuclear structural changes appears functionally significant and independent of the mitotic signaling pathway," *J. Cellular Biochemistry* **70**: 130 – 140 (1998).